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Reactivity of Deoxynivalenol (Vomitoxin) Monoclonal Antibody Towards Putative Trichothecene Precursors and Shunt Metabolites

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ABSTRACT

The reactivity of deoxynivalenol (DON) monoclonal antibody 6F5 towards trichothecenes and *Fusarium* metabolites associated with DON and T-2 toxin biosynthesis was assessed by indirect enzyme-linked immunosorbent assay. Using concentrations required to inhibit antibody binding to a DON hemisuccinate ovalbumin conjugate solid phase by 50% as a basis of comparison, isotrichodermin, 3-acetyl DON, 15-deacetylcalonectrin, and 3,15-dideacetylcalonectrin were 647, 362, 247, and 209% cross-reactive relative to DON, respectively. Isotrichodermol, sambucinol, and 7,8-dihydroxycalonectrin reacted to a lesser extent. The results suggested that the C3, C4, and C15 positions were immunodominant on determining binding to the DON monoclonal antibody. The antibody should be useful in detecting both production of these metabolites in *Fusarium* cultures as well as their occurrence in foods and feeds.

The trichothecenes are a group of naturally occurring sesquiterpenoid metabolites that are produced by several fungi including *Fusarium*. Trichothecenes include some of the most potent protein synthesis inhibitors known (*13*) and two of these, deoxynivalenol (DON, vomitoxin) and T-2 toxin, have been associated with feed refusal, emesis, and immunosuppression. A number of trichothecenes and metabolites produced in *Fusarium* cultures has been identified as putative precursors or shunt products in the biosyntheses of DON and T-2 toxin (*2,5,9,10,14*). Many of these are structurally based on the modification of the parent 12,13-epoxytrichothecene-9-ene skeleton with oxygen functions including esters and alcohols at one or more positions (C3, C4, C7, C8, and C15) (Fig. 1). The degree of natural occurrence of these metabolites and their potential toxicity is generally unknown. In one instance, Kim et al. (*7*) reported that 3,15-dideacetylcalonectrin (isoverrucarol) was produced by *Fusarium oxysporum* in wheat cultures and that it exhibited substantially greater toxicity than DON.

Although trichothecene detection is generally based on thin layer chromatography, gas chromatography, or mass spectroscopy, it is now possible to use immunochemical assays. Notably enzyme-linked immunosorbent assays (ELISAs) have been developed for detection of mycotoxins in various agricultural commodities (*11*). A major challenge for the trichothecenes has been to develop suitable immunogens for antibody production. Recently we described the production of a monoclonal antibody-based ELISA for DON (*3*). Here, the C7 and C15-hydroxyls (a 1,3 diol) were protected with a cyclic boronate ester prior to esterification with a cyclic anhydride. This allowed derivatization of DON exclusively at C3 and enabled conjugation to a carrier protein and production of a monoclonal antibody that reacts with DON. Since many trichothecenes share common structural moieties with DON, it is possible that these also react with the above-described antibody. In this report we determined reactivity of the DON monoclonal antibody for selected metabolites associated with trichothecene biosynthesis and identify key functional groups which contribute to its specificity.

MATERIALS AND METHODS

Reagents

All inorganic chemicals and organic solvents were reagent grade or better. Ovalbumin (OA) (crude and fraction VII), polyethylene sorbitan monolaurate (Tween 20), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide, pristane, N-hydroxysuccinimide, dimethylformamide (DMF), and 1,3-dicyclohexylcarbodiimide were purchased from Sigma Chemical Co. (St. Louis, MO). Dullbecco modified Eagle's medium, penicillin/streptomycin solution, NCTC supplemental medium, sodium pyruvate, and fetal bovine serum were from Gibco Laboratories (Grand Island, NY). Mice were purchased from Charles River Laboratories (Wilmington, MA). Goat antimouse IgG conjugated to horseradish peroxidase was obtained from Cooper Biomedical (Malvern, PA). Deoxynivalenol (DON) and 3-acetyl-DON were purchased from Romer Labs, Inc. (Washington, MO). Trichodiene, 15-deacetylcalonectrin, 3,15-dideacetylcalonectrin, isotrichodermin, and isotrichodermol were prepared as previously described by McCormick et al. (*10*); 15-acetyl DON was prepared as described previously (*12*). Sambucinol and 7,8-dihydroxycalonectrin were graciously supplied by D.J Miller (Chemistry and Biology Research Institute, Agriculture Canada, Ottawa), and 4,15-diacetylnivalenol was kindly provided by T. Yoshizawa (Kagawa University, Japan).

Deoxynivalenol-hemisuccinate (DON-HS) was prepared by protection of the C7- and C15-hydroxyls with a cyclic boronate ester, esterification at the C3-position, and then removal of the boronate ester (*3*). In a 2-ml reaction vial, 10 mg DON was

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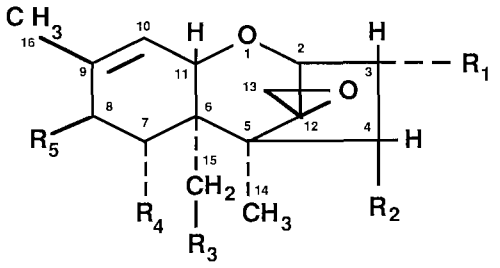


Compound	R_1	R_2	R_3	R_4	R_5
Deoxynivalenol	OH	H	OH	OH	=O
Isotrichodermin	OAc[a]	H	H	H	H_2
3-Acetyl-DON	OAc	H	OH	OH	=O
15-Deacetylcalonectrin	OAC	H	OH	H	H_2
3,15-Dideacetylcalonectrin	OH	H	OH	H	H_2
Isotrichodermol	OH	H	H	H	H_2
7,8-Dihydroxycalonectrin	OAc	H	OAc	OH	OH
15-Acetyl-DON	OH	H	OAc	OH	=O
4,15-Diacetylnivalenol	OH	OAc	OAc	OH	=O
Nivalenol	OH	OH	OH	OH	=O
Fusarenon-X	OH	OAc	OH	OH	=O
T-2 Toxin	OH	OAc	OAc	H	ISV[b]
Verrucarol	H	OH	OH	H	H_2
Trichodermin	H	OAc	H	H	H_2

[a] OAc = -OOCCH 3 [b] ISV= -OOCCH2 CH (CH3)2

Figure 1. *Structures of putative trichothecene precursors and shunt metabolites.*

dissolved in 0.5 ml pyridine, then 7 mg of n-butyl boronate was added and the mixture was reacted at room temperature with stirring for 30 min. Succinic anhydride (14 mg) was added, the vials capped tightly and heated in a boiling water bath for 45 min. Pyridine was evaporated under N_2 and the product dissolved in 2 ml H_2O. The aqueous solution was washed with chloroform, extracted 10 times with 5 ml ethyl ether, and the ether fraction evaporated under N_2. The DON-HS was conjugated to ovalbumin using N-hydroxysuccinimide and 1,3-dicyclohexylcarbodiimide as described by Kitagawa et al. (*8*).

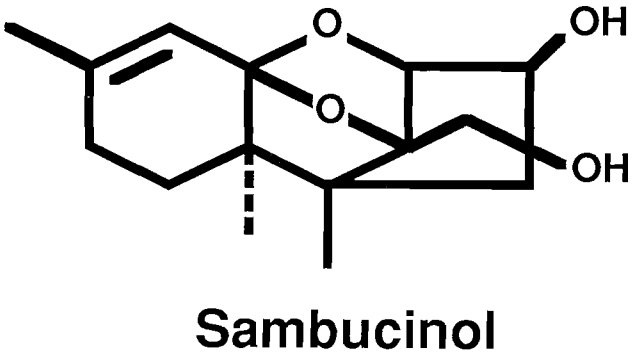
Hybridoma cell line 6F5 which secretes anti-DON monoclonal antibody was produced as ascites (*4*), and the antibody was purified by ammonium sulfate precipitation (*6*).

Indirect competitive ELISA

Microtiter plates (Immunolon-2, Dynatech Laboratories, Alexandria, VA) were coated overnight (4°C) with 100 µl per well of DON-HS-OA (5 µg/ml) in 0.01 M carbonate buffer (pH 9.6). The plates were washed four times with 0.01 M phosphate-buffered saline (PBS, pH 7.2) containing 0.05% (v/v) Tween-20 (PBS-Tween). To each well was added 300 µl of 1% (w/v) ovalbumin in PBS for 30 min to block the unbound sites of solid phase and to minimize nonspecific binding. After washing four times with PBS-Tween, 50 µl of DON (or other metabolites tested) was added with 50 µl of the appropriate dilution of DON monoclonal antibody. Plates were incubated for 60 min at 37°C and wells washed six times as described above. Bound antibody was then determined after the addition of antimouse IgG peroxidase conjugate (100 µl/well of 1:500 in 1% OA PBS) and the resultant mixture incubated for 30 min. After the wells were washed eight times, bound peroxidase was determined with ABTS substrate as described by Abouzied et al. (*1*).

RESULTS AND DISCUSSION

The reactivity of the DON monoclonal antibody was determined in an indirect ELISA using DON hemisuccinate ovalbumin conjugate as the solid phase. The capacity of selected trichothecenes (Fig. 1) and biosynthetically related metabolites (Fig. 2) to inhibit binding of the antibody to the solid phase was compared to that found for free DON (Fig. 3). It was notable that the concentration of isotrichodermin,



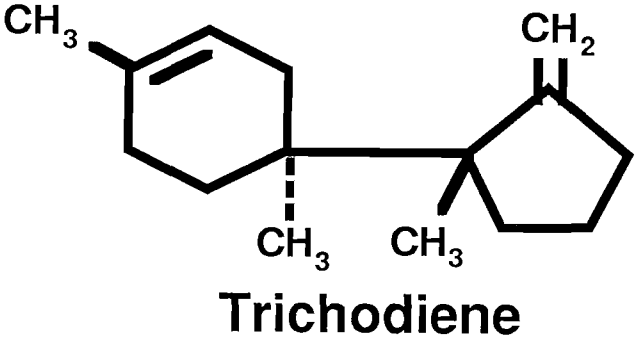


Figure 2. *Structures of trichodiene and sambucinol.*

3-acetyl DON, 15-deacetylcalonectrin, and 3,15-dideacetylcalonectrin required to inhibit antibody binding by 50% were 210, 375, 550, and 650 ng/ml, respectively, whereas that for DON was 1360 ng/ml (Table 1). Apparently the presence of an acetyl group at C3 (Fig. 1) in isotrichodermin, 3-acetyl DON, and 15-deacetylcalonectrin was a requisite for the comparatively enhanced antibody reactivity. Substitution of the acetoxy group at C3 by a hydroxyl as occurs in 3,15-dideacetylcalonectrin resulted in slightly decreased reactivity relative to DON (209%) compared to 15-deacetylcalonectrin (247%). Similarly, there was a marked decrease in relative reactivity by hydroxyl substitution at C3 for an acetoxy group as was evident in comparing isotrichodermin (647%) and isotrichodermol (74%) as well as 3-acetyl DON (362%) and DON (100%).

Compounds with reactivity for the monoclonal antibody appeared to have in common a hydrogen at the C4 variable

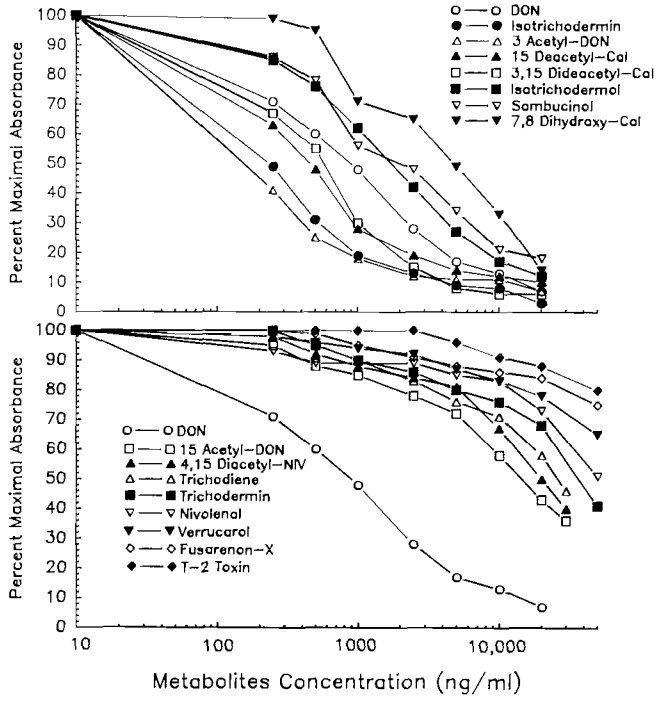


Figure 3. *Reactivity of DON monoclonal antibody 6F5 towards* Fusarium *metabolites as assessed by indirect ELISA.*

TABLE 1. *Specificity of monoclonal antibody for deoxynivalenol in competitive indirect ELISA.*

Mycotoxins	50% inhibition (ng/ml)	Cross-reactivity[a] (%)
Deoxynivalenol (DON)	1360	100
Isotrichodermin	210	647
3-acetyl-DON	375	362
15-deacetylcalonectrin	550	247
3,15-dideacetylcalonectrin	650	209
Isotrichodermol	1850	74
Sambucinol	2000	68
7,8-dihydroxycalonectrin	5100	27
15-acetyl-DON	17,000	8
4,15-diacetylnivalenol	21,500	6
Trichodiene	26,000	5
Trichodermin	37,000	4
Nivalenol	>50,000	<3
Verrucarol	>50,000	<3
Fusarenon-X	>50,000	<3
T-2 Toxin	>50,000	<3

[a]Cross-reactivity defined as (ng/ml of DON required for 50% inhibition/ng/ml of analogue required for 50% inhibition) x 100.

position (Fig. 1; Table 1) with the exception of 4,15-diacetylnivalenol. The recognition of the C15 position by the antibody was also apparent. Replacement of the C15 hydroxy of DON with an acetoxy group as occurs in 15-acetyl DON resulted in a reduction from 100% to 8% relative reactivity. A similar relationship was observed when binding 3-acetyl-DON (647%) was compared to 7,8-dihydroxycalonectrin (27%).

Although it does not have a C12-C13 epoxide, sambucinol was significantly bound by the antibody. This was likely favored by the moieties present at C3, C4, and C15. Relatedly, we have previously determined that the DON monoclonal antibody does not recognize the absence of the C12-C13 epoxide in deepoxy DON (*3*). Finally, trichodiene, the biosynthetic precursor of both the trichothecenes and sambucinol, showed only 5% cross-reactivity.

In summary, monoclonal antibody 6F5 exhibited a high degree reactivity for several *Fusarium* metabolites in addition to DON. Critical moieties which were immunodominant were located at the C3, C4, and C15 positions. This antibody should be especially useful in screening for both production of isotrichodermin, DON, 3-acetyl DON, 15-deacetylcalonectrin, 3,15-dideacetylcalonectrin, isotrichodermin, and sambucinol by *Fusarium* isolates as well as the occurrence of these metabolites in food and feed samples. Where specific identification of compounds that cause inhibition in immunoassays employing this antibody is desired, subsequent quantitation and confirmation by a second method will be necessary.

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REFERENCES

1. Abouzied, M.M., A. Asghar, M.A. Pearson, J.I. Gray, E.R. Miller, and J.J. Pestka. 1990. Monoclonal antibody-based enzyme-linked immunosorbent assay for C_{19}-Δ^{16}-steroids in sera of boar pigs. J. Agric. Food Chem. 38:331-335.
2. Baldwin, N.C.P., B.W. Bycroft, P.M. Dewick, and D.C. March. 1987. Trichothecene mycotoxins from *Fusarium culmorum* cultures. Z. Naturforsch. 42c:1043-1049.
3. Casale, W.L., J.J. Pestka, and L.P. Hart. 1988. Enzyme-linked immunosorbent assay employing monoclonal antibody specific for deoxynivalenol (vomitoxin) and several analogues. J. Agric. Food Chem. 36:663-668.
4. Goding, J.W. 1983. Monoclonal antibodies: Principles and practice. Academic Press, New York.
5. Greenhalgh, R., D. Levandier, W. Adams, J.D. Miller, B.A. Blackwell, A.J. McAlees, and A. Taylor. 1986. Production and characterization of deoxynivalenol and other secondary metabolites of *Fusarium culmorum* (CMI 14764, HLX 1503). J. Agric. Food Chem. 34:98-102.
6. Hebert, G.A., P.L. Pelhman, and B. Pittman. 1973. Determination of the optimum ammonium sulfate concentration for the fractionation of rabbit, sheep, horse, and goat antisera. Appl. Microbiol. 37:26-36.
7. Kim, K.-H., Y.-W. Lee, C.J. Mirocha, and R.J. Pawlosky. 1990. Isoverrucarol production by *Fusarium oxysporum* CJS-12 isolated from corn. Appl. Environ. Microbiol. 56:260-263.
8. Kitagawa, T., T. Shimozona, T. Yoshida, and H. Nishimura. 1981. Preparation and characterization of heterobifunctional cross linking reagents for protein modifications. Chem. Pharm. Bull. 29:1130-1135.
9. Lauren, D.R., A. Ashley, B.A. Blackwell, R. Greenhalgh, J.D. Miller, and G.A. Neish. 1987. Trichothecenes produced by *Fusarium crookwellense* DAOM 193611. J. Agric. Food Chem. 35:884-889.
10. McCormick, S.P., S.L. Taylor, R.D. Plattner, and M. Beremand. 1989. New modified trichothecenes accumulated in solid culture by mutant strains of *Fusarium sporotrichioides*. Appl. Environ. Microbiol. 55:2195-2199.
11. Pestka, J.J. 1988. Enhanced surveillance of foodborne mycotoxins by immunochemical assay. J. Assoc. Off. Anal. Chem. 71:1075-1081.
12. Pestka, J.J., W.S. Lin, and J.H. Forsell. 1986. Decreased feed consumption and body weight gain in the B6C3Fl mouse after dietary exposure to 15-acetyldeoxynivalenol. Food Chem. Toxicol. 24:1309-1315.
13. Ueno, Y. 1977. Mode of action of trichothecenes. Pure Appl. Chem. 49:1737-1745.
14. Zamir, L.O. 1989. Biosynthesis of 3-acetyldeoxynivalenol and sambucinol. Tetrahedron 45:2277-2305.